WHAT IS CLAIMED:

1. A method of detecting single nucleotide polymorphisms comprising:

providing a sample potentially containing a target nucleic acid molecule;

subjecting the sample to a polymerase chain reaction process involving use of oligonucleotide amplification primers under conditions effective to amplify any of the target nucleic acid molecule present in the sample to produce an amplification product;

subjecting the amplification product to treatment with a phosphatase under conditions effective to remove 5' phosphates from free deoxynucleotide triphosphates (dNTPs) in the amplification product;

inactivating the phosphatase;

providing an oligonucleotide extension primer complementary to a portion of the target nucleic acid molecule;

providing a nucleic acid polymerizing enzyme;

providing a plurality of types of nucleotide analogs;

blending the amplification product treated with a phosphatase, the oligonucleotide extension primer, the nucleic acid polymerizing enzyme, and the nucleotide analogs, each type being present in a first amount, to form an extension solution where the oligonucleotide extension primer is hybridized to the target nucleic acid molecule to form a primed target nucleic acid molecule and the nucleic acid polymerizing enzyme is positioned to add nucleotide analogs to the primed target nucleic acid molecule at an active site;

extending the oligonucleotide extension primer in the extension solution by using the nucleic acid polymerizing enzyme to add a nucleotide analog to the oligonucleotide extension primer at the active site to form an extended oligonucleotide extension primer, wherein the nucleotide analog being added is complementary to the nucleotide of the target nucleic acid molecule at the active site;

determining the amounts of each type of the nucleotide analogs in the extension solution after said extending, each type being present in a second amount;

comparing the first and second amount of each type of the nucleotide analog; and

identifying the type of nucleotide analog where the first and second amounts differ as the nucleotide added to the oligonucleotide extension primer at the active site.

2. A method according to claim 1, wherein the target nucleic acid molecule is present in the sample as double stranded DNA.

3. A method according to claim 1, wherein the target nucleic acid molecule is present in the sample as single stranded DNA.

4. A method according to claim 1, wherein the target nucleic acid molecule is present in the sample as RNA.

5. A method according to claim 1, wherein said oligonucleotide amplification primer and said oligonucleotide extension primer have different melting temperatures (T_m).

6. A method according to claim 1, wherein the phosphatase is calf intestinal alkaline phosphatase, shrimp alkaline phosphatase, and mixtures thereof.

7. A method according to claim 1, wherein said subjecting the sample to a polymerase chain reaction process involves use of oligonucleotide amplification primers, said method further comprising:

digesting the oligonucleotide amplification primers which do not produce the amplification product during said subjecting the sample to a polymerase chain reaction process with exonuclease I and

inactivating the exonuclease I.

8. A method according to claim 1, wherein said subjecting the sample to a polymerase chain reaction process involves use of oligonucleotide amplification primers, one of which contains a 5'-phosphate group, said method

further comprising:

subjecting the amplification product, prior to said subjecting the amplification product to treatment with a phosphatase, with λ-exonuclease under conditions effective to digest one strand of the amplification product containing the 5'-phosphate group; and

inactivating the λ-exonuclease.

9. A method according to claim 1, wherein each type of nucleotide analog is a dideoxy nucleotide analog.

10. A method according to claim 1, wherein said determining is carried out by electrospraying the extension solution.

11. A method according to claim 10, wherein said electrospraying is carried out with an electrospray device comprising:

a substrate having an injection surface and an ejection surface opposing the injection surface, wherein the substrate is an integral monolith comprising:

an entrance orifice on the injection surface;

an exit orifice on the ejection surface;

a channel extending between the entrance orifice and the exit orifice; and

a recess extending into the ejection surface and surrounding the exit orifice, thereby defining a nozzle on the ejection surface.

12. A method according to claim 11, wherein said substrate has a plurality of entrance orifices on the injection surface, a plurality of exit orifices on the ejection surface with each of the plurality of exit orifices corresponding to a respective one of the plurality entrance orifices, and a plurality of channels extending between one of the plurality of exit orifices and the corresponding one of the plurality of entrance orifices.

13. A method according to claim 11, wherein the electrospray device further comprises:

a voltage application system comprising:

a first electrode attached to said substrate to impart a first potential to said substrate and

a second electrode to impart a second potential, wherein the first and the second electrodes are positioned to define an electric field surrounding the exit orifice.

14. A method according to claim 13, wherein the first electrode is electrically insulated from fluid passing through said electrospray device and the second potential is applied to the fluid.

15. A method according to claim 13, wherein the first electrode is in electrical contact with fluid passing through said electrospray device and the second electrode is positioned on the ejection surface.

16. A method according to claim 13, wherein application of potentials to said first and second electrodes causes fluid passing through said electrospray device fluid to discharge from the exit orifice in the form of a spray.

17. A method according to claim 13, wherein application of potentials to said first and second electrodes causes fluid passing through said electrospray device fluid to discharge from the exit orifice in the form of droplets.

18. A method according to claim 11, wherein said electrospray device further comprises:

a porous polymeric material associated with said electrospray device at a location suitable to effect liquid chromatographic separation of materials passing through said electrospray device.

19. A method according to claim 10, wherein said determining further comprises detecting the amounts of each type of the nucleotide analogs in the electrospray.

20. A method according to claim 19, wherein said detecting is carried out by mass spectrometry, fluorescence, ion conductivity, liquid chromatography, capillary electrophoresis, radioactive assay, NMR, ELISA, and combinations thereof.

21. A method according to claim 10 further comprising:

passing the extension solution through a metal chelating resin prior to said electrospraying.

22. A method according to claim 1, wherein said method is carried out in a single container.

23. A method of detecting single nucleotide polymorphisms comprising:

providing a sample potentially containing a target nucleic acid molecule;

subjecting the sample to a polymerase chain reaction process involving use of oligonucleotide amplification primers under conditions effective to amplify any of the target nucleic acid molecule present in the sample to produce an amplification product;

passing the amplification product through a molecular weight filter configured to retain amplified target nucleic acid molecule but not the amplification primers;

providing an oligonucleotide extension primer complementary to a portion of the target nucleic acid molecule;

providing a nucleic acid polymerizing enzyme;

providing a plurality of types of nucleotide analogs;

blending the retained target nucleic acid molecule, the oligonucleotide extension primer, the nucleic acid polymerizing enzyme, and the

nucleotide analogs, each type being present in a first amount, to form an extension solution where the oligonucleotide extension primer is hybridized to the target nucleic acid molecule to form a primed target nucleic acid molecule and the nucleic acid polymerizing enzyme is positioned to add nucleotide analogs to the primed target nucleic acid molecule at an active site;

extending the oligonucleotide extension primer in the extension solution by using the nucleic acid polymerizing enzyme to add a nucleotide analog to the oligonucleotide extension primer at the active site to form an extended oligonucleotide extension primer, wherein the nucleotide analog being added is complementary to the nucleotide of the target nucleic acid molecule at the active site;

determining the amounts of each type of the nucleotide analogs in the extension solution after said extending, each type being present in a second amount;

comparing the first and second amount of each type of the nucleotide analog; and

identifying the type of nucleotide analog where the first and second amounts differ as the nucleotide added to the oligonucleotide extension primer at the active site.

24. A method according to claim 23, wherein the target nucleic acid molecule is present in the sample as double stranded DNA.

25. A method according to claim 23, wherein the target nucleic acid molecule is present in the sample as single stranded DNA.

26. A method according to claim 23, wherein the target nucleic acid molecule is present in the sample as RNA.

27. A method according to claim 23, wherein said oligonucleotide amplification primer and said oligonucleotide extension primer have different melting temperatures (T_m).

28. A method according to claim 23, wherein said subjecting the

sample to a polymerase chain reaction process involves use of oligonucleotide amplification primers, one of which contains a 5'-phosphate group, said method further comprising:

subjecting the amplification product, prior to said passing the amplification product through a molecular weight filter, with λ-exonuclease under conditions effective to digest one strand of the amplification product containing the 5'-phosphate group; and

inactivating the λ-exonuclease.

29. A method according to claim 23, wherein said passing the amplification product is vaccum-assisted.

30. A method according to claim 23, wherein each type of nucleotide analog is a dideoxy nucleotide analog.

31. A method according to claim 23 wherein said determining is carried out by electrospraying the extension solution.

32. A method according to claim 31, wherein said electrospraying is carried out with an electrospray device comprising:

a substrate having an injection surface and an ejection surface opposing the injection surface, wherein the substrate is an integral monolith comprising:

an entrance orifice on the injection surface;

an exit orifice on the ejection surface;

a channel extending between the entrance orifice and the exit orifice; and

a recess extending into the ejection surface and surrounding the exit orifice, thereby defining a nozzle on the ejection surface.

33. A method according to claim 32, wherein said substrate has a plurality of entrance orifices on the injection surface, a plurality of exit orifices on the ejection surface with each of the plurality of exit orifices corresponding to a

respective one of the plurality entrance orifices, and a plurality of channels extending between one of the plurality of exit orifices and the corresponding one of the plurality of entrance orifices.

34. A method according to claim 32, wherein the electrospray device further comprises:

a voltage application system comprising:

a first electrode attached to said substrate to impart a first potential to said substrate; and

a second electrode to impart a second potential, wherein the first and the second electrodes are positioned to define an electric field surrounding the exit orifice.

35. A method according to claim 34, wherein the first electrode is electrically insulated from fluid passing through said electrospray device and the second potential is applied to the fluid.

36. A method according to claim 34, wherein the first electrode is in electrical contact with fluid passing through said electrospray device and the second electrode is positioned on the ejection surface.

37. A method according to claim 34, wherein application of potentials to said first and second electrodes causes fluid passing through said electrospray device fluid to discharge from the exit orifice in the form of a spray.

38. A method according to claim 34, wherein application of potentials to said first and second electrodes causes fluid passing through said electrospray device fluid to discharge from the exit orifice in the form of droplets.

39. A method according to claim 32, wherein said electrospray device further comprises:

a porous polymeric material associated with said electrospray device at a location suitable to effect liquid chromatographic separation of materials passing through said electrospray device.

40. A method according to claim 31, wherein said determining further comprises detecting the amounts of each type of the nucleotide analogs in the electrospray.

41. A method according to claim 40, wherein said detecting is carried out by mass spectrometry, fluorescence, ion conductivity, liquid chromatography, capillary electrophoresis, radioactive assay, NMR, ELISA, and combinations thereof.

42. A method according to claim 31 further comprising:

passing the extension solution through a metal chelating resin prior to said electrospraying.

43. A method according to claim 23, wherein said method is carried out in a single container.

44. A method of detecting single nucleotide polymorphisms comprising:

providing a sample potentially containing a target nucleic acid molecule;

subjecting the sample to a polymerase chain reaction process involving use of oligonucleotide amplification primers under conditions effective to amplify any of the target nucleic acid molecule present in the sample to produce an amplification product;

subjecting the amplification product to treatment with a phosphatase under conditions effective to remove 5' phosphates from free dNTPs in the amplification product;

inactivating the phosphatase;

providing an oligonucleotide extension primer complementary to a portion of the target nucleic acid molecule;

providing a nucleic acid polymerizing enzyme;

providing a plurality of types of nucleotide analogs;

blending the amplification product treated with a phosphatase, the oligonucleotide extension primer, the nucleic acid polymerizing enzyme, and the nucleotide analogs to form an extension solution where the oligonucleotide extension primer is hybridized to the target nucleic acid molecule to form a primed target nucleic acid molecule and the nucleic acid polymerizing enzyme is positioned to add nucleotide analogs to the primed target nucleic acid molecule at an active site;

extending the oligonucleotide extension primer in the extension solution by using the nucleic acid polymerizing enzyme to add a nucleotide analog to the oligonucleotide extension primer at the active site to form an extended oligonucleotide extension primer, wherein the nucleotide analog being added is complementary to the nucleotide of the target nucleic acid molecule at the active site; and

determining the nucleotide analog added to the oligonucleotide extension primer at the active site by electrospraying the extended oligonucleotide extension primer.

45. A method according to claim 44, wherein the target nucleic acid molecule is present in the sample as double stranded DNA.

46. A method according to claim 44, wherein the target nucleic acid molecule is present in the sample as single stranded DNA.

47. A method according to claim 44, wherein the target nucleic acid molecule is present in the sample as RNA.

48. A method according to claim 44, wherein said oligonucleotide amplification primer and said oligonucleotide extension primer have different melting temperatures (T_m).

49. A method according to claim 44, wherein the phosphatase is calf intestinal alkaline phosphatase, shrimp alkaline phosphatase, and mixtures thereof.

50. A method according to claim 44, wherein said subjecting the sample to a polymerase chain reaction process involves use of oligonucleotide amplification primers, said method further comprising:

digesting the oligonucleotide amplification primers which do not produce the amplification product during said subjecting the sample to a polymerase chain reaction process with exonuclease I and

inactivating the exonuclease I.

51. A method according to claim 44, wherein said subjecting the sample to a polymerase chain reaction process involves use of oligonucleotide amplification primers, one of which contains a 5'-phosphate group, said method further comprising:

subjecting the amplification product with λ-exonuclease, prior to said subjecting the amplification product to treatment with a phosphatase, under conditions effective to digest one strand of the amplification product containing the 5'-phosphate group; and

inactivating the λ-exonuclease.

52. A method according to claim 44, wherein each type of nucleotide analog is a dideoxy nucleotide analog.

53. A method according to claim 44, wherein said electrospraying is carried out with an electrospray device comprising:

a substrate having an injection surface and an ejection surface opposing the injection surface, wherein the substrate is an integral monolith comprising:

an entrance orifice on the injection surface;

an exit orifice on the ejection surface;

a channel extending between the entrance orifice and the exit orifice; and

a recess extending into the ejection surface and surrounding the exit orifice, thereby defining a nozzle on the ejection surface.

54. A method according to claim 53, wherein said substrate has a plurality of entrance orifices on the injection surface, a plurality of exit orifices on the ejection surface with each of the plurality of exit orifices corresponding to a respective one of the plurality entrance orifices, and a plurality of channels extending between one of the plurality of exit orifices and the corresponding one of the plurality of entrance orifices.

55. A method according to claim 53, wherein the electrospray device further comprises:

a voltage application system comprising:

a first electrode attached to said substrate to impart a first potential to said substrate and

a second electrode to impart a second potential, wherein the first and the second electrodes are positioned to define an electric field surrounding the exit orifice.

56. A method according to claim 55, wherein the first electrode is electrically insulated from fluid passing through said electrospray device and the second potential is applied to the fluid.

57. A method according to claim 55, wherein the first electrode is in electrical contact with fluid passing through said electrospray device and the second electrode is positioned on the ejection surface.

58. A method according to claim 55, wherein application of potentials to said first and second electrodes causes fluid passing through said electrospray device fluid to discharge from the exit orifice in the form of a spray.

59. A method according to claim 55, wherein application of potentials to said first and second electrodes causes fluid passing through said electrospray device fluid to discharge from the exit orifice in the form of droplets.

60. A method according to claim 53, wherein said electrospray device further comprises:

a porous polymeric material associated with said electrospray device at a location suitable to effect liquid chromatographic separation of materials passing through said electrospray device.

61. A method according to claim 44, wherein said determining further comprises detecting the amounts of each type of the nucleotide analogs in the electrospray.

62. A method according to claim 61, wherein said detecting is carried out by mass spectrometry, fluorescence, ion conductivity, liquid chromatography, capillary electrophoresis, MALDI-TOF, radioactive assay, NMR, ELISA, and combinations thereof.

63. A method according to claim 44 further comprising:

passing the extension solution through a molecular weight filter configured to separate the amplified target nucleic acid molecule and the extended oligonucleotide extension primers from low molecular weight reaction components, prior to said electrospraying.

64. A method according to claim 63, wherein said passing the extension solution is vacuum-assisted.

65. A method according to claim 44, wherein said method is carried out in a single container.

66. A method of detecting single nucleotide polymorphisms comprising:

providing a sample potentially containing a target nucleic acid molecule;

subjecting the sample to a polymerase chain reaction process involving use of oligonucleotide amplification primers under conditions effective to amplify any of the target nucleic acid molecule present in the sample to produced an amplification product;

passing the amplification product through a molecular weight filter configured to retain amplified target nucleic acid molecule but not the amplification primers;

providing an oligonucleotide extension primer complementary to a portion of the target nucleic acid molecule;

providing a nucleic acid polymerizing enzyme;

providing a plurality of types of nucleotide analogs;

blending the retained target nucleic acid molecule, the oligonucleotide extension primer, the nucleic acid polymerizing enzyme, and the nucleotide analogs to form an extension solution where the oligonucleotide extension primer is hybridized to the target nucleic acid molecule to form a primed target nucleic acid molecule and the nucleic acid polymerizing enzyme is positioned to add nucleotide analogs to the primed target nucleic acid molecule at an active site;

extending the oligonucleotide extension primer in the extension solution by using the nucleic acid polymerizing enzyme to add a nucleotide analog to the oligonucleotide extension primer at the active site to form an extended oligonucleotide extension primer, wherein the nucleotide analog being added is complementary to the nucleotide of the target nucleic acid molecule at the active site; and

determining the nucleotide analog added to the oligonucleotide extension primer at the active site by electrospraying the extended oligonucleotide extension primer.

67. A method according to claim 66, wherein the target nucleic acid molecule is present in the sample as double stranded DNA.

68. A method according to claim 66, wherein the target nucleic acid molecule is present in the sample as single stranded DNA.

69. A method according to claim 66, wherein the target nucleic acid molecule is present in the sample as RNA.

70. A method according to claim 66, wherein said oligonucleotide amplification primer and said oligonucleotide extension primer have different melting temperatures (T_m).

71. A method according to claim 66, wherein said subjecting the sample to a polymerase chain reaction process involves use of oligonucleotide amplification primers, one of which contains a 5'-phosphate group, said method further comprising:

subjecting the amplification product, prior to said passing the amplification product through a molecular weight filter, with λ-exonuclease under conditions effective to digest one strand of the amplification product containing the 5'-phosphate group; and

inactivating the λ-exonuclease.

72. A method according to claim 66, wherein said passing the amplification product is vacuum-assisted.

73. A method according to claim 66, wherein each type of nucleotide analog is a dideoxy nucleotide analog.

74. A method according to claim 66, wherein said electrospraying is carried out with an electrospray device comprising:

a substrate having an injection surface and an ejection surface opposing the injection surface, wherein the substrate is an integral monolith comprising:

an entrance orifice on the injection surface;

an exit orifice on the ejection surface;

a channel extending between the entrance orifice and the exit orifice; and

a recess extending into the ejection surface and surrounding the exit orifice, thereby defining a nozzle on the ejection surface.

75. A method according to claim 74, wherein said substrate has a plurality of entrance orifices on the injection surface, a plurality of exit orifices on the ejection surface with each of the plurality of exit orifices corresponding to a respective one of the plurality entrance orifices, and a plurality of channels extending between one of the plurality of exit orifices and the corresponding one of the plurality of entrance orifices.

76. A method according to claim 74, wherein the electrospray device further comprises:

a voltage application system comprising:

a first electrode attached to said substrate to impart a first potential to said substrate and

a second electrode to impart a second potential, wherein the first and the second electrodes are positioned to define an electric field surrounding the exit orifice.

77. A method according to claim 76, wherein the first electrode is electrically insulated from fluid passing through said electrospray device and the second potential is applied to the fluid.

78. A method according to claim 76, wherein the first electrode is in electrical contact with fluid passing through said electrospray device and the second electrode is positioned on the ejection surface.

79. A method according to claim 76, wherein application of potentials to said first and second electrodes causes fluid passing through said electrospray device fluid to discharge from the exit orifice in the form of a spray.

80. A method according to claim 76, wherein application of potentials to said first and second electrodes causes fluid passing through said electrospray device fluid to discharge from the exit orifice in the form of droplets.

81. A method according to claim 74, wherein said electrospray device further comprises:

a porous polymeric material associated with said electrospray device at a location suitable to effect liquid chromatographic separation of materials passing through said electrospray device.

82. A method according to claim 66, wherein said determining further comprises detecting the amounts of each type of the nucleotide analogs in the electrospray.

83. A method according to claim 82, wherein said detecting is carried out by mass spectrometry, fluorescence, ion conductivity, liquid chromatography, capillary electrophoresis, MALDI-TOF, radioactive assay, NMR, ELISA, and combinations thereof.

84. A method according to claim 66 further comprising:

passing the extension solution through a metal chelating resin prior to said electrospraying.

85. A method according to claim 66 further comprising:

passing the extension solution through a molecular weight filter configured to separate the amplified target nucleic acid molecule and the extended oligonucleotide extension primers from low molecular weight reaction components, prior to said electrospraying.

86. A method according to claim 85, wherein said passing the extension solution is vacuum-assisted.

87. A method according to claim 66, wherein said method is carried out in a single container.